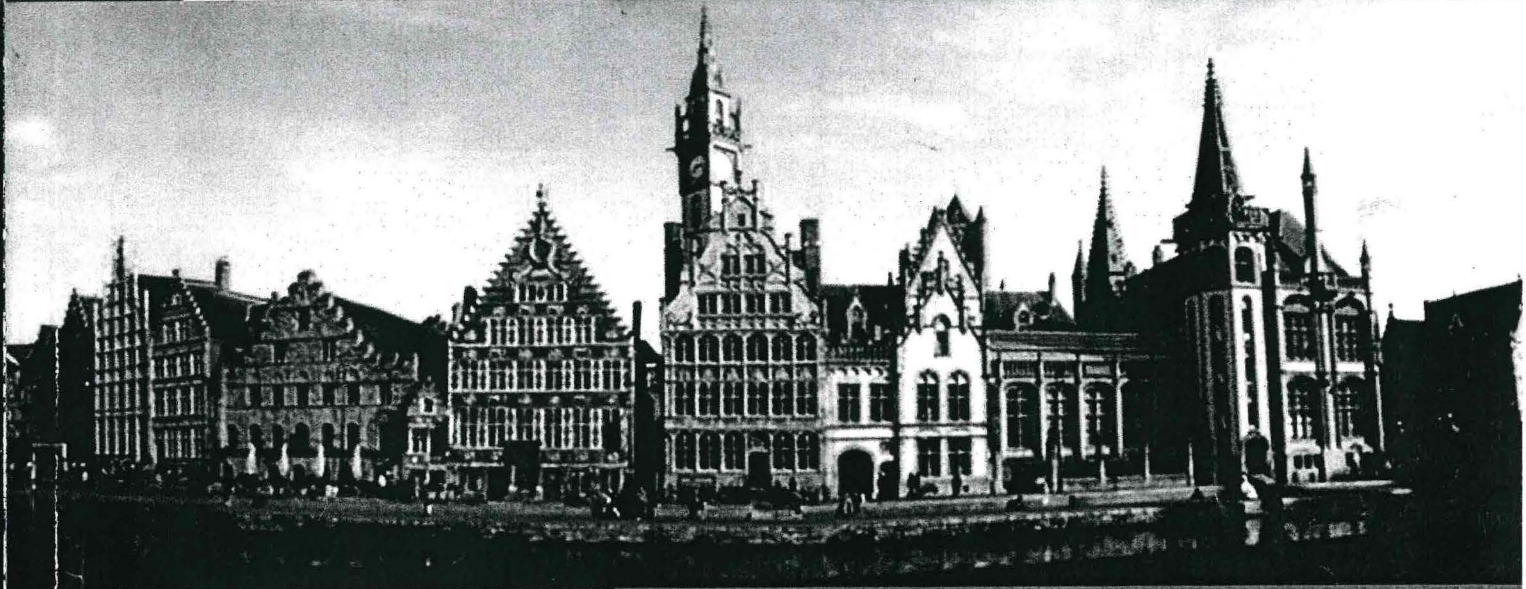

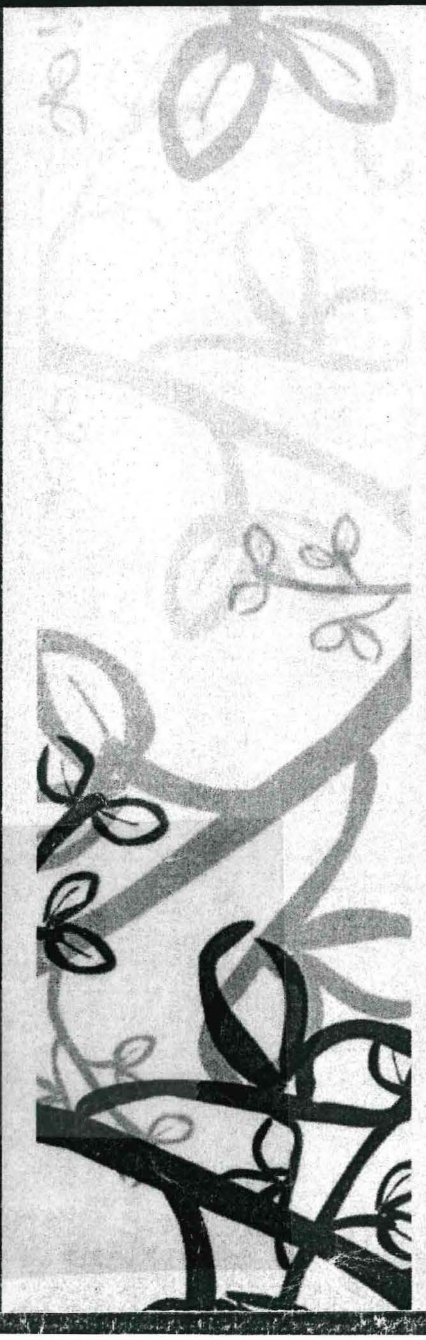

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Biotechnological advances in *in vitro* horticultural breeding

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PROGRAM
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Session 4: Micropropagation and regeneration

DEVELOPMENT OF NEW VARIETAL TYPES BASED ON REJUVENATION BY SOMATIC EMBRYOGENESIS AND PROPAGATION BY CONVENTIONAL BUDDING OR MICROCUTTINGS IN *HEVEA BRASILIENSIS*

Montoro, P.[1], Carron, M.P.[1], Granet, F.[2], Lardet, L.[1], Leclercq, J.[1], Dessailly, F.[1], Martin, F.[1], Gaurel, S.[2], Uche, E.[3], Rio, M.[1] and Oliver, G.[1]

[1]*UMR AGAP, CIRAD, 34398 Montpellier, France*
[2]*CPN, Michelin, 63040 Clermont-Ferrand, France*
[3]*RENL, Benin City, Edo State, Nigeria*

Hevea brasiliensis has been the only commercial source of natural rubber for a century. This plant species, which is planted on more than 10 million hectares, is of major importance for the economies of producing countries in Southeast Asia and West Africa. Cloning by budding on rootstocks derived from illegitimate seedlings made it possible to select the best individuals and obtain substantial genetic gain in the 1940s compared to seedling plantations. From the 1970s to date, the development of micropropagation techniques has not led to any commercial application for the multiplication of self-rooted clones. However, some serious leads have been advanced for achieving large-scale multiplication of improved material in the medium term, using biotechnologies.

Although their multiplication rate is low, microcuttings and primary somatic embryogenesis techniques produce better quality planting material than budded clones. Conversely, maintained somatic embryogenesis gives a higher multiplication rate, but several developmental parameters of the *in vitro* plantlets are affected. A combination of embryogenic callus cryopreservation and indirect secondary somatic embryogenesis has made it possible to reduce the length of time calli are exposed to hormones and the number of proliferation cycles. Although the quality of the *in vitro* plantlets has been improved, the maintained embryogenesis pathway involving callus multiplication remains problematic. Combining primary somatic embryogenesis to rejuvenate the planting material and multiplication by budding or microcuttings seems to be an alternative for improving the planting material. Rejuvenated budded clones have been planted on 80 hectares by CIRAD and Michelin. These trials reveal better budding and growth success for four clones compared to conventional budded clones confirming previous results from small-scale trials on eleven clones. At the same time, a team at CATAS has demonstrated the merits of microcuttings from emblings. Besides, a highly efficient transgenesis technique has been developed for *Hevea* using maintained embryogenic friable callus. Although the quality of the planting material is affected by the regeneration of plants using maintained somatic embryogenesis, this technique remains an essential tool for functional genomics. Functional analyses have been launched for several genes such as transcription factors, an ethylene receptor and genes involved in the detoxification of activated oxygen species.

These three decades of work on rubber tree micropropagation have resulted in primary somatic embryogenesis being considered as an efficient and true-type rejuvenation technique. The rejuvenation of 11 rubber tree clones has led to the establishment of rejuvenated budwood gardens on an agronomy trial scale. This has also resulted in an awareness of the role played by juvenility in planting material vigour and the need to maintain it in the budwood gardens of new selected clones. Maintained somatic embryogenesis entails a risk of somaclonal variation. Based on the results obtained for other woody species recalcitrant to micropropagation, an alternative using direct secondary somatic embryogenesis might alleviate the problem. Lastly, cloning own-rooted plants offers major prospects for the selection and multiplication of rootstocks, which will have a substantial future impact in a context of adaptation to climate change.